ABSTRACT

Name of Sponsor/Company	Janssen-Cilag International NV*
Name of Finished Product	INVOKANA® (canagliflozin), VOKANAMET® (canagliflozin/metformin)
Name of Active Ingredient(s)	JNJ-28431754 (canagliflozin)

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Protocol No.: 28431754NAP4001

Title of Study: Meta-Analysis of Amputation Events from Clinical Trials DIA3008 (CANVAS), DIA4003 (CANVAS-R), and DNE3001 (CREDENCE) (Version 3.0, 25 March 2019)

Sponsor's Responsible Medical Officer: Norman Rosenthal, MD

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Study Center(s): Not applicable

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Background and Rationale: Diabetes mellitus, a chronic medical condition, is associated with significant morbidity and mortality. One of the more devastating outcomes of diabetic complications is amputation of the lower limb. Estimates suggest that people with diabetes had a risk of lower limb amputation that was 10 to 20 times higher than in nondiabetics.

The canagliflozin cardiovascular (CV) outcomes clinical program (CANVAS Program) consists of 2 CV outcome studies: 28431754DIA3008 (CANagliflozin cardioVascular Assessment Study, ie, CANVAS) and 28431754DIA4003 (CANagliflozin cardioVascular Assessment Study – Renal, ie, CANVAS-R). More than 10,000 randomized subjects with type 2 diabetes mellitus (T2DM) and either CV disease (secondary prevention) or at least 2 risk factors for a CV event (primary prevention) were included in the CANVAS Program. Data from the CANVAS Program showed an approximately 2-fold increased risk of atraumatic lower limb amputation (an excess of 3 events per 1,000 patient-years) in subjects at high CV risk who were treated with canagliflozin compared with placebo. Based on the review of the data from the CANVAS Program, the Pharmacovigilance Risk Assessment Committee (PRAC) considered that treatment with canagliflozin contributes to an increased risk of amputation of the lower limb, mainly of the toe. However, the PRAC also noted that the mechanism of action currently is not well understood and considered that the risk of lower limb amputation may constitute a possible class effect (Article 20 Referral EMEA/H/A-20/1442).

The CREDENCE study (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation Trial; 28431754DNE3001) was a renal outcome study that included

4,401 randomized subjects with T2DM, Stage 2 or 3 chronic kidney disease, and macroalbuminuria, who were receiving standard of care therapy including a maximum tolerated labeled daily dose of an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker. There was no increased risk in lower limb amputation from treatment with canagliflozin observed in the CREDENCE study.

As part of the pharmacovigilance activities agreed to during the Article 20 Referral, the marketing authorization holder was required to conduct a meta-analysis of the canagliflozin CV and renal outcome studies after all data were available as a category 3 study in the Risk Management Plan (RMP). The PRAC considered that the meta-analysis should include 1) a graph of the cumulative incidence of amputation events over time with the number of patients "at risk" at different time points and 2) a graph of the cumulative incidence of potential precursor adverse events of interest over time with the number of patients "at risk" at different time points.

Research Question and Objectives: This study was intended to estimate the relative risk of the time to first occurrence of atraumatic lower limb amputation and to evaluate risk factors for lower limb amputation in patients at high risk for CV events and/or progression of kidney disease who were treated with canagliflozin compared with placebo, pooled across 3 studies (CANVAS, CANVAS-R, and CREDENCE).

Objectives

Primary Objective

To estimate the relative risk of the time to first occurrence of atraumatic lower limb amputation for canagliflozin relative to placebo (ie, hazard ratio [HR] and 95% confidence interval [CI]) in the various patient populations in a meta-analysis pooled across the CANVAS, CANVAS-R, and CREDENCE studies.

Secondary Objective

To evaluate the risk of preceding adverse events of interest that *potentially* led to atraumatic lower limb amputation in different study groups (canagliflozin compared with placebo), including an analysis of risk factors defined by patient (ie, baseline characteristics) in subgroups of patients.

Hypothesis

No hypotheses were prespecified. This was a Post Authorization Safety Study (PASS) that aims to estimate the relative risk of the time to first occurrence of amputation and evaluate risk factors for amputation in patients treated with canagliflozin and who were at high risk for CV events and/or progression of kidney disease.

Study Design: This was a retrospective, non-interventional PASS to characterize the risk for lower limb amputation events in patients who had been treated with canagliflozin compared with placebo, through a meta-analysis of clinical trial data. The meta-analysis was conducted with individual patient data based on a 1-step approach.

Setting: This was a retrospective analysis of clinical trial data from specified clinical trials (CANVAS, CANVAS-R, and CREDENCE) as requested by the Committee for Medicinal Products for Human Use in the RMP for canagliflozin.

Patient Population and Study Size: Data from canagliflozin- or placebo-treated subjects in the 3 completed outcome trials (CANVAS, CANVAS-R, and CREDENCE) were evaluated. The patient population of CANVAS and CANVAS-R was subjects with T2DM and either CV disease (secondary prevention) or at least 2 risk factors for a CV event (primary prevention). The patient population of CREDENCE were subjects with T2DM, Stage 2 or 3 chronic kidney disease, and macroalbuminuria, who were receiving standard of care therapy including a maximum tolerated labeled daily dose of an

angiotensin-converting enzyme inhibitor or angiotensin receptor blocker. Subjects in the CANVAS study were randomized to 1 of 3 parallel arms of canagliflozin 100 mg, canagliflozin 300 mg, or matching placebo, whereas subjects in the CANVAS-R study were randomized to 1 of 2 parallel arms of canagliflozin 100 mg or matching placebo, up-titrated to 300 mg or matching placebo, based on investigator assessment. Subjects in the CREDENCE study were randomized to 1 of 2 parallel arms of canagliflozin 100 mg or matching placebo.

The CANVAS and CANVAS-R studies were conducted in a combined total of 667 sites in 30 countries. The CREDENCE study was conducted in 690 sites in 34 countries. In the meta-analysis dataset, 7,990 subjects have been treated with canagliflozin and 6,541 subjects have been treated with placebo, for a total of 14,531 subjects.

Variables and Data Sources: Baseline demographics, baseline risk factors, and exposure were evaluated. All lower limb amputations from the 3 completed outcome trials (CANVAS, CANVAS-R, and CREDENCE) for all subjects who had received either canagliflozin or placebo were evaluated. Preceding adverse events of interest that potentially led to amputation were evaluated.

The data sources are the databases for the completed canagliflozin clinical trials (CANVAS, CANVAS-R, and CREDENCE). No other electronic case report form specific for this study was used.

Statistical Methods:

For assessment of cross-study heterogeneity, a Cox proportional hazards model including treatment as an explanatory factor, study (ie, CANVAS, CANVAS-R, and CREDENCE), and the treatment-by-study interaction was fit. If significant statistical heterogeneity was identified (ie, the p-value for the interaction was ≤ 0.10), a Gail-Simon test was to be performed to assess the nature of the interaction (ie, quantitative or qualitative). A forest plot of the HR and corresponding 95% confidence intervals was provided by study for visual inspection of the degree of cross-study variability. Additional heterogeneity statistics, such as the I-squared statistic, were also provided.

The relative risk of the time to first occurrence of amputation for canagliflozin compared with placebo was estimated (HR and 95% confidence interval) in the On-treatment analysis set (and repeated in the On-study analysis set as an additional sensitivity analysis) using a stratified Cox proportional hazards regression model including a term for treatment (canagliflozin compared with placebo) as the explanatory variable and stratified by study (ie, CANVAS, CANVAS-R, and CREDENCE). A Kaplan-Meier plot for the time to first occurrence of amputation was provided by treatment group for the pooled data (and similarly for each study) and displayed the number of patients “at risk” at relevant time points.

To account for the competing risk of mortality, a Fine & Gray subdistribution hazard model was fit and the amputation-specific cumulative incidence was estimated.

Baseline risk factor analyses based on the protocol-defined subgroups were performed by testing the corresponding treatment-by-subgroup interaction through the addition of this term and the risk factor as covariates to the primary model.

Post-treatment adverse events (serious adverse events and/or adverse events leading to discontinuation) that may be associated with amputation were evaluated. For each adverse event group (ie, vascular adverse events, diabetic foot-related adverse events, wound and infection, nervous system disorders, volume depletion), the total number of events, the total amount of observed subject-years, as well as the corresponding event rates per 1,000 subject-years were summarized by treatment group. The relative risk of the time to first occurrence of “conditions leading to amputation” for canagliflozin compared with placebo (HR and 95% confidence interval) was estimated using the same stratified Cox proportional hazards regression model described above. A corresponding Kaplan-Meier plot for the time to first occurrence of each adverse event grouping was also provided by treatment group.

RESULTS:

PARTICIPANTS AND PATIENT CHARACTERISTICS: All treated subjects (who received at least 1 dose of study drug) in the CANVAS, CANVAS-R, and CREDENCE studies were included in the meta-analysis. Across the pooled studies, the overall mean duration of exposure to study drug (canagliflozin or placebo) was 138.76 weeks. The total exposure to study drug was 22,905.3 subject-years in the combined canagliflozin group and 15,737.8 subject-years in the placebo group. The overall mean follow-up (on- or off-treatment) was 172.49 weeks, with the majority of subjects (>96%) having a follow-up of more than 78 weeks.

Demographic and baseline characteristics were generally similar across groups. The mean age of subjects was 63.2 years and approximately one-third of the subjects were women. Approximately 75% of the subjects were white, ~15% identified as Asians, and ~4% as black or African-American; approximately 21% of all subjects were of Hispanic or Latino ethnicity. The distribution of subjects enrolled across geographic regions was 24.8% in North America, 30.7% in Europe, 13.5% in Central/South America, and 30.9% in the rest of world.

In both the combined canagliflozin and the placebo groups, subjects had a mean duration of diabetes of about 14 years and a baseline mean hemoglobin A_{1c} (HbA_{1c}) of 8.25% and a mean body mass index of 31.8 kg/m^2. Similar proportions of subjects in both groups had a history of CV disease (61%, and 21.7% had peripheral vascular disease), a history of diabetic neuropathy (36.1%), a history of amputation (3.2%), and baseline use of diuretics (45%). The mean estimated glomerular filtration rate (eGFR) level at baseline was approximately 70 $mL/min/1.73m^2$, with 30.7% of subjects at an eGFR level of 30 to <60 $mL/min/1.73m^2$ and 49.4% at 60 to <90 $mL/min/1.73m^2$.

MAIN RESULTS:

Cross-Study Heterogeneity

While there was no significant cross-study heterogeneity observed (p=0.1531) in the On-treatment analysis set based on a 2-sided significance level of 0.10, a moderate degree of heterogeneity was observed based on the I^2 statistic of 44%, which suggests that nearly half of the observed heterogeneity can be ascribed to variation across studies.

As suggested and informed by the potential cross-study heterogeneity observed in the On-treatment analysis set, subsequent analyses in the On-study set were also performed. Significant cross-study heterogeneity was observed in the On-study analysis set (p=0.0557) and further confirmed by the I^2 statistic of 65%, which suggests that nearly two-thirds of the observed heterogeneity can be ascribed to variation across studies.

Incidence and Location

All lower limb amputations except that in 1 subject were atraumatic. The overall pattern of distribution by amputation location was similar between canagliflozin and placebo groups. The majority of amputations were minor (ie, toe and trans-metatarsal) amputations, with toes being the most common amputation site, occurring in 71.4% (125 of 175 subjects with an amputation) and 82.1% (69 of 84 subjects with an amputation) in the canagliflozin and placebo groups, respectively. The pattern of distribution of amputations by highest anatomical location (where each subject was counted only once, regardless of leg) was also similar between the canagliflozin and placebo groups, except trans-metatarsal amputations, which had a modestly higher incidence rate in the canagliflozin group compared with placebo (1.23 and 0.43 per 1,000 subject-years, respectively).

Risk of Atraumatic Amputation and Time to Occurrence

The primary analysis of the time to the first occurrence of amputation was based on a stratified Cox proportional hazards model including treatment as the explanatory variable and was stratified by study. As shown in the table below, the incidence rate for atraumatic lower limb amputations was 7.54 per 1,000 subject-years in the combined canagliflozin group and 5.21 per 1,000 subject-years in the placebo group, with an HR of canagliflozin versus placebo of 1.59 (95% CI: 1.22, 2.07).

Hazard Ratio of Atraumatic Lower Limb Amputation (Study 28431754-NAP4001: On-Treatment Analysis Set)

	Placebo (N=6541)	Cana (N=7990)
Subjects with any event, n(%)	84 (1.3)	175 (2.2)
Number of events	118	244
Subject-year of exposure to first event	16134.5	23210.9
Incidence rate(/1000 subject-years) (a)	5.21	7.54
IRD(/1000 subject-years) (minus Placebo) (b)		2.33(0.75, 3.92)
HR (vs. Placebo) (c)		1.59(1.22, 2.07)

Note: (a) Incidence is based on the number of subjects with at least one amputation and not number of events.
Incidence rates are per 1000 subject-years, where a subject's exposure time is calculated from Day 1 to the first amputation event date.
Note: (b) 95% CI is based on Normal approximation for the incidence rate difference (IRD).
Note: (c) Hazard ratio (HR) is from a stratified Cox proportional hazards model including treatment as the explanatory variable, and stratified by study.
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The Kaplan-Meier plot for the first occurrence of atraumatic lower limb amputation over time in the On-treatment analysis set showed that a separation between the canagliflozin and placebo cumulative amputation incidence curves was seen within the first 26 weeks of treatment initiation.

The same analysis was also performed as a sensitivity analysis in the On-study analysis set. The incidence rate for atraumatic lower limb amputations was similar to those obtained in the On-treatment analysis set, with 7.53 per 1,000 subject-years in the combined canagliflozin group and 5.62 per 1,000 subject-years in the placebo group, and an HR of canagliflozin versus placebo of 1.50 (95% CI: 1.19, 1.89).

Risk of Atraumatic Amputation and Time to Occurrence After Adjusting for Competing Risk of Mortality

After adjusting for the competing risk of all-cause mortality based on the Fine & Gray subdistribution model, the HR (the combined canagliflozin group versus the placebo group) for atraumatic lower limb amputation is nearly identical to the primary analysis, with an HR of canagliflozin versus placebo of 1.60 (95% CI: 1.23, 2.07), indicating that there was no impact from the competing risk of all-cause mortality on the estimation of amputation risk.

OTHER ANALYSES:

Risk of Amputation by Dosage

For subjects randomized to receive canagliflozin 100 mg only in CANVAS and CREDENCE studies, a pooled randomized sensitivity analysis for comparison between canagliflozin 100 mg and placebo was conducted. The incidence rate for atraumatic lower limb amputations was 8.52 per 1,000 subject-years in the canagliflozin 100 mg group and 5.78 per 1,000 subject-years in the placebo group, with an HR of canagliflozin versus placebo of 1.50 (95% CI: 1.09, 2.06).

Due to the dose titration in the CANVAS-R study and the lack of the canagliflozin 300 mg group in the CREDENCE study, a randomized analysis by dose across all 3 studies cannot be performed.

The only randomized dose analysis with both 100 mg and that 300 mg dosing was performed using data from the CANVAS study. No apparent dose-response relationship was observed, with the HR of canagliflozin 100 mg versus placebo being 2.30 (95% CI: 1.31, 4.03), and the HR of canagliflozin 300 mg versus placebo being 2.02 (95% CI: 1.14, 3.58).

Baseline Risk Factor Analyses

The forest plot for the HRs and 95% CIs of atraumatic lower limb amputation by predefined subgroups was created. The only significant interaction of treatment-by-subgroup based on the Cox proportional hazards model, including treatment, baseline subgroup and their interaction, was baseline HbA_{1c} (p=0.0525). For subjects who had a baseline HbA_{1c} <8%, the HR was 1.08 (95% CI: 0.66,1.75), and for subjects who had a baseline HbA_{1c} ≥8%, the HR was 1.84 (95% CI: 1.34, 2.53). There was no qualitative interaction of treatment-by-subgroup by baseline HbA_{1c} based on the Gail-Simon test (p=0.500).

ADVERSE EVENTS/ADVERSE REACTIONS *POTENTIALLY* LEADING TO AMPUTATION:

Only serious adverse events and adverse events that led to study drug discontinuation were systematically collected in all 3 studies and thus summarized.

Among the 5 predefined groupings of adverse events, the only grouping with an incidence rate per 1,000 subject-years that was higher (ie, the 95% confidence interval excluding 1) in the combined canagliflozin group (10.47 per 1,000 subject-years) compared to placebo (8.94 per 1,000 subject-years) was the diabetic foot-related grouping (HR: 1.31; 95% CI: 1.07, 1.62). This grouping included terms such as diabetic foot infection, skin ulcers, osteomyelitis, and gangrene, which are known precursor conditions for amputation.

Hazard Ratios of AEs Potentially Leading to Amputation by AE Category - Serious AEs or AEs Leading to Study Drug Discontinuation (Study 28431754-NAP4001: On-Treatment Analysis Set)

	---------- Placebo ----------		---------- Cana ----------		
Endpoint	n/N(%)	EVRT[a]	n/N(%)	EVRT[a]	HR[b] (95% CI)
Vascular Adverse Events	78/6541 (1.2)	4.81	142/7990 (1.8)	6.04	1.27 (0.96, 1.69)
Diabetic Foot-Related	145/6541 (2.2)	8.94	246/7990 (3.1)	10.47	1.31 (1.07, 1.62)
Wound and Infection	22/6541 (0.3)	1.36	39/7990 (0.5)	1.66	1.25 (0.73, 2.12)
Nervous System Disorders	6/6541 (0.1)	0.37	7/7990 (0.1)	0.30	0.96 (0.32, 2.89)
Volume Depletion	18/6541 (0.3)	1.11	13/7990 (0.2)	0.55	0.53 (0.26, 1.10)
Total exposure in subject-year	16227.3		23502.9		

Note: [a] Event rates are per 1000 subject-years. The denominator is the total of each subject's exposure of the study medication plus 30 days.
Note: [b] Hazard ratio (canagliflozin compared to placebo) and its 95% CI are estimated using a stratified Cox proportional hazards model including treatment as the explanatory variable, and stratified by study.
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To eliminate the confounding effect of having an amputation on the analyses of precursor events, the above analyses were repeated in subjects without an on-treatment amputation. There were no differences in the groupings of adverse events between the combined canagliflozin group and the placebo group, with the 95% confidence intervals including 1 in all 5 adverse event groupings. The lack of an imbalance in the incidence of adverse events potentially leading to amputation in subjects who did not experience an amputation suggests that canagliflozin does not increase the incidence of these conditions.

Hazard Ratios of AEs Potentially Leading to Amputation by AE Category - Serious AEs or AEs Leading to Study Drug Discontinuation for Subjects without On-treatment Amputation (Study 28431754-NAP4001: On-Treatment Analysis Set)

	---------- Placebo ----------		---------- Cana ----------		
Endpoint	n/N(%)	EVRT[a]	n/N(%)	EVRT[a]	HR[b] (95% CI)
Vascular Adverse Events	64/6457 (1.0)	4.00	103/7815 (1.3)	4.49	1.14 (0.83, 1.56)
Diabetic Foot-Related	79/6457 (1.2)	4.93	107/7815 (1.4)	4.67	1.09 (0.81, 1.46)
Wound and Infection	13/6457 (0.2)	0.81	25/7815 (0.3)	1.09	1.45 (0.74, 2.87)
Nervous System Disorders	6/6457 (0.1)	0.37	7/7815 (0.1)	0.31	0.97 (0.32, 2.91)
Volume Depletion	17/6457 (0.3)	1.06	13/7815 (0.2)	0.57	0.57 (0.27, 1.18)
Total exposure in subject-year	16017.7		22917.9		

Note: [a] Event rates are per 1000 subject-years. The denominator is the total of each subject's exposure of the study medication plus 30 days.
Note: [b] Hazard ratio (canagliflozin compared to placebo) and its 95% CI are estimated using a stratified Cox proportional hazards model including treatment as the explanatory variable, and stratified by study.
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DISCUSSION AND CONCLUSION:

DISCUSSION:

This is a retrospective meta-analysis using pooled data from 3 completed clinical outcome studies that have key differences in patient population (such as baseline amputation rate), post-baseline amputation incidence rate (represented by the rate in the placebo group), and risk of amputation associated with canagliflozin treatment. The CREDENCE population had a higher baseline rate and post-baseline incidence rate for atraumatic lower limb amputation as expected for this study population than those in the CANVAS and CANVAS-R studies. While a higher risk of lower limb amputation with canagliflozin was seen in CANVAS and CANVAS-R studies, it was not observed in the CREDENCE study. Due to the significant cross-study heterogeneity, the pooled data should be interpreted with caution.

In the pooled On-treatment analysis set of the 3 studies, treatment with canagliflozin was associated with an increased risk for atraumatic lower limb amputations with an HR of 1.59 (95% CI: 1.22, 2.07), driven by the data from CANVAS and CANVAS-R studies. The incidence rate difference between canagliflozin and placebo and the resulting HR in this meta-analysis were not unexpected when data from the CANVAS and CANVAS-R studies (with a higher risk observed for canagliflozin) and the CREDENCE study (without a higher risk observed for canagliflozin) were pooled together. The estimated risk using pooled data underestimates the risk in the CANVAS (HR=2.16 [95% CI: 1.28, 3.65]) and CANVAS-R (HR=1.84 [95% CI: 1.09, 3.10]) population and overestimates the risk in the CREDENCE (HR=1.20 [95% CI: 0.81, 1.78]) population.

A randomized analysis by dose across all 3 studies could not be performed due to the dose titration in the CANVAS-R study and the lack of the canagliflozin 300 mg group in CREDENCE study. A randomized dose analysis including the canagliflozin 100 mg and 300 mg groups was previously performed in the CANVAS study and that showed no apparent dose-response relationship.

Conditions potentially leading to amputation in the predefined group of diabetic foot-related adverse events, which include terms such as diabetic foot infection, skin ulcers, osteomyelitis, and gangrene, occurred more frequently in subjects in the canagliflozin group compared with placebo in this pooled analysis. This observation was not unexpected since the incidence of amputations was higher in the canagliflozin group in the CANVAS and CANVAS-R studies, and such precursor events are likely to track with the risk of amputations.

However, in subjects who did not develop an on-treatment amputation during these studies, no meaningful differences between canagliflozin and placebo groups in the incidence rate of precursor events for amputations were observed. These data suggest that canagliflozin does not increase the risk of

developing a condition that potentially leads to an amputation, but may increase the chance of having an amputation once a precursor condition is already present.

Of note, in the CREDENCE study, treatment with canagliflozin was not associated with an increased risk in lower limb amputation or of adverse events considered as potential precursors to amputation compared with placebo. The reason for this cross-study difference remains unclear, but the data add to the evidence of heterogeneity across the 3 studies in this meta-analysis.

CONCLUSIONS:

Significant heterogeneity on the risk of amputation was observed across the 3 studies, therefore, caution should be taken when interpreting these pooled results. Pooled data from the 3 studies showed canagliflozin was associated with an approximately 1.6-fold risk of lower limb amputation, driven by the risk observed from CANVAS and CANVAS-R studies. There was no increased risk of amputation associated with canagliflozin treatment observed in the CREDENCE study. Canagliflozin did not increase the incidence of the conditions potentially leading to amputation.